Journal of Pioneering Medical Sciences
Received: June 04, 2025 | Accepted: October 03, 2025 | Published: November 05, 2025
Volume 14, Issue 10, Pages 43-51

DOI https://doi.org/10.47310/jpms2025141006

Time-Dependence Effect of 2.45 GHz RF-EMR Exposure on Male Reproductive Hormones and LHCGR

Norazurashima Jamaludin[1], Siti Fatimah Ibrahim[2], Khairul Osman[3], Syed Muhamad Asyraf Syed Taha[4], Atikah Hairulazam[5], Sivasatyan Vijay[6], Aini Farzana Zulkefli[7], Mohd Farisyam Mat Ros[8] and Farah Hanan Fathihah Jaffar[9*]

[1,3]Center for Diagnostic, Therapeutic and Investigative Studies (CODTIS), Faculty of Health Sciences, Universiti Kebangsaan Malaysia (UKM), Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

[2,4-9]Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM), Cheras 56000 Kuala Lumpur, Malaysia

[1]Department of Anatomy and Physiology, Institut Latihan Kementerian Kesihatan Malaysia (ILKKM), Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia

Author Designation: [1-3,9]Lecturer, [8]Researcher

*Corresponding author: Farah Hanan Fathihah Jaffar (e-mail: farahhanan@ukm.edu.my).

©2025 the Norazurashima Jamaludin *et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0

Abstract As wireless technologies become increasingly integrated into daily life, concerns have grown regarding the biological effects of Radiofrequency Electromagnetic Radiation (RF-EMR), particularly its influence on the Luteinizing Hormone (LH)–LH receptor (LHCGR)-testosterone pathway within the Hypothalamic-Pituitary-Gonadal (HPG) axis. This study investigated the effects of 2.45 GHz Wi-Fi exposure on this pathway in male rats. A total of 24 male Sprague Dawley rats were divided into four groups (n = 6 per group) and exposed to Wi-Fi for 0 (control), 4, 8, or 24 hours daily for eight weeks. Serum LH and testosterone levels were measured by ELISA. LHCGR gene and protein expression were assessed using RT-qPCR and Western blot. All data were analyzed using ANOVA, except for serum testosterone levels which were analyzed using the Kruskal-Wallis test. No significant differences were found in systemic LH or testosterone levels between groups. However, LHCGR mRNA expression showed a significant time-dependent increase. LHCGR protein levels decreased with shorter exposure durations and showed partial improvement at 24 hours, though they remained significantly lower than controls. These findings suggest that although systemic hormonal levels remain stable, RF-EMR exposure may associated with molecular alterations in testicular tissue, including compensatory upregulation of LHCGR expression. The increased LHCGR gene expression, alongside the fluctuating protein levels, indicates an adaptive, but not fully restorative response in Leydig cells compared with non-exposed controls.

Key Words LHCGR, Testosterone Regulation, Wi-Fi, HPG Axis, Male Fertility

INTRODUCTION

In today's digitally connected world, wireless technologies have become an inseparable part of daily life. Wi-Fi networks, especially those operating at the 2.45 GHz frequency, enable seamless communication and data access, whether at home, in the workplace, or in public spaces. In Malaysia, daily Wi-Fi usage typically falls into three categories: mild (<4 hours), intermediate (5-12 hours) and heavy (>15 hours). These usage patterns guided our choice of exposure durations (4, 8 and 24 hours) to reflect realistic daily conditions [1]. While these advancements offer significant convenience, they expose individuals to continuous radiofrequency electromagnetic radiation (RF-EMR), raising concerns about potential long-term health implications [2].

One area of growing concern is the impact of RF-EMR on the male reproductive system. This concern is particularly relevant given the widespread habit of carrying mobile devices in trouser pockets, positions radiation sources near the testes, potentially increasing the risk of reproductive harm due to prolonged, low-level exposure [3,4]. Even when not actively being used, mobile devices stay connected to Wi-Fi, constantly emitting radiofrequency signals as they communicate with base stations and run background apps [5,6]. Over time, this persistent emission at the commonly used 2.45 GHz frequency contributes to chronic RF-EMR exposure in everyday settings.

Several studies have observed reductions in serum testosterone and LH levels following RF-EMR exposure, suggesting that endocrine balance may be particularly vulnerable to these subtle but continuous signals [7,8,9]. Testosterone is critical for developing and maintaining male reproductive tissues and physiological functions, while LH

stimulates Leydig cells in the testes to produce testosterone [10,11,12]. Disruption in this hormonal balance may impair spermatogenesis, lower libido and negatively impact fertility. Some reports have also indicated alterations in Follicle-Stimulating Hormone (FSH), pointing to broader dysfunction of the HPG axis [8,9].

Although these hormonal disturbances have been consistently reported, the mechanisms underlying RF-EMR-induced endocrine disruption remain poorly defined. As of today, previous study had elucidated that oxidative stress can impair Leydig cell steroidogenesis by reducing enzymes activity and altering receptor signaling, while neurotransmitter imbalance at the hypothalamic level may dysregulate GnRH release and downstream LH secretion [9]. In addition, RF-EMR has been shown to influence intracellular signaling pathways such as MAPK and PKA, which regulate steroidogenic enzymes and receptor sensitivity, suggesting further molecular mechanisms that could disrupt the LH–LHCGR–testosterone axis [4]. Together, these mechanisms suggest that RF-EMR could disrupt the LH–LHCGR–testosterone pathway both at systemic and cellular levels [9].

Despite these findings, current literature shows inconsistencies. A few studies report hormonal changes, whereas others find no effect. These variations are often due to differences in experimental design, such as exposure frequency, duration, sample size, or reliance only on systemic hormone measurements without examining molecular endpoints [8,11,13]. Methodological variation also plays a role, including differences in dosimetry reporting (SAR values), exposure setup and near field conditions, all of which can alter tissue-specific absorption.

For instance, exposures at 900 MHz for periods ranging from 30 minutes to 2 hours daily often showed reductions in testosterone and decreased LH levels [14-17]. However, [18] reported no significant changes in LH level, while [19] observed an increase in LH despite reduced testosterone. On the other hand, daily exposures at 1800 MHz led to reductions in testosterone and LH [20,21]. Similarly, [22] reported a reduction in testosterone following daily exposure to 2.45 GHz RF-EMR for 30 days. Regardless the various applied RF-EMR frequency, the previous reports indicate that RF-EMR-induced hormonal disruption may originate from interference with the endocrine system, affecting hormonal regulation and physiological function within the male reproductive axis [22,23].

Given the discrepancies in testosterone and LH findings in previous studies, this study aimed to evaluate the time-dependent effects of 2.45 GHz Wi-Fi exposure on the:

- Serum LH and testosterone levels
- LHCGR gene expression between groups
- LHCGR protein expression between groups

By addressing both the duration of exposure and the hormone–receptor interaction, our findings may help explain the inconsistencies observed in earlier studies. It is hypothesized that prolonged Wi-Fi exposure would alter LHCGR expression at the molecular level, even in the absence of systemic changes in LH or testosterone, reflecting a compensatory adaptation in Leydig cells. Understanding this pathway may provide a deeper comprehension of the underlying mechanisms and contribute to more effective mitigation strategies to address fertility-related risks of RF-EMR exposure.

METHODS

Animals

A total of 24 healthy male Sprague Dawley rats, 8 weeks old and weighing 200 to 250 grams were obtained from the Universiti Kebangsaan Malaysia (UKM) Animal House Facility, which maintains animals in Specific-Pathogen-Free (SPF) conditions. The animals were housed under controlled environmental conditions, with a room temperature maintained at 22±3°C and a standard 12-hour light/dark cycle. They were fed a commercial pellet diet supplied by UKM and had access to water ad libitum. Body weight was monitored weekly during the 8-week exposure period. After a week of acclimatization, the rats were allocated into four equal groups using a simple randomization method (n = 6 per group): one control group and three experimental groups. The sample size was determined with reference to OECD TG 420 and supported by U.S. EPA guidance (712-C-026, 2012), as no specific guideline exists for RF-EMR exposure in rodents.

Each rat was housed in a plastic cage (29 × 43 × 16 cm) to minimize external influences and inter-animal interference. For the experimental groups, exposure was conducted using a TP-LINK AC750 Wireless Dual Band Wi-Fi Router (model Archer C20, Shenzhen, China), which emitted RF-EMR at 2.45 GHz, conforming to the IEEE 802.11n Wi-Fi standard. The cages were positioned at a consistent distance of 20 cm from the router to ensure uniform exposure. The SAR was estimated to be 0.41 W/kg as the same setting was implies based on the previous study [25]. The experimental room was internally lined with aluminum foil to isolate it from external RF-EMR sources and prevent signal interference. Rats in the experimental groups were continuously exposed to Wi-Fi radiation for eight weeks. At the end of the exposure period, all animals were euthanized via an intraperitoneal injection of a cocktail consisting of Ketamine, Xylazine and Zoletil. Death was confirmed by the absence of the righting reflex, lack of heartbeat, no response to tail pinch, no withdrawal reflexes in both forelimbs and hindlimbs and the absence of a corneal reflex. Following euthanasia, blood serum and testicular tissues were collected for further analysis. Euthanasia and sample collection were conducted in the morning hours (between 8:00 and 10:00 am) to minimize variation due to diurnal rhythms. All procedures involving animals, including euthanasia, were reviewed and approved by the Animal Ethics Committee of UKM (Approval Number: FSK/2022/KHAIRULOSMAN/20-JULY/1262-AUG.-2022-AUG.-2024) and conducted by institutional animal care guidelines.

Study Groups

Following a one-week acclimatization period, the animals were randomly assigned into four groups (n = 6 per group) and subjected to different durations of Wi-Fi exposure over 8 weeks, as outlined below:

- **Control:** Non-operational 2.45 GHz Wi-Fi device
- **Test 1:** 2.45 GHz Wi-Fi device for 4 hours per day
- **Test 2:** 2.45 GHz Wi-Fi device for 8 hours per day
- **Test 3:** 2.45 GHz Wi-Fi device continuously for 24 hours daily

Blood Serum

Following euthanasia, blood samples were collected via cardiac puncture and transferred into plain blood collection tubes. The samples were incubated at 37°C for 30 minutes to allow clotting. Subsequently, they were centrifuged at 1500×g for 10 minutes at 4°C. The resulting serum was carefully extracted, transferred into labelled Eppendorf tubes and stored at −80°C until further analysis.

Testes Collection

The testes of each rat were dissected and any residual blood was thoroughly removed. Gauze and pre-warmed Phosphate-Buffered Saline (PBS) was used to clean the tissue gently. The testes were immersed in liquid nitrogen and stored at −80°C for further analysis.

Testosterone and LH by ELISA

An ELISA kit was used to measure the concentrations of LH and testosterone in the previously prepared serum samples. All procedures were carried out according to the manufacturer's instructions (Elabscience, Wuhan, China). The ELISA plates were then read using a SpectraMax Plus 384 Microplate Spectrophotometer (Molecular Devices, California, USA) at a wavelength of 450 nm. The coefficient of variation (CV) for all ELISA kits' intra-assay and interassay variability was below 10%. The levels of testosterone and LH in the serum were determined by interpolating from a standard curve of 8 points. The curve was produced using a 4-parameter logistic (4 PL) model and the analysis was performed using MyAssays.com.

Determination of LH Receptor Gene Expression Using Reverse Transcription-Quantitative Real-Time PCR (RT-qPCR)

Approximately 30 mg of testicular tissue was isolated for RNA extraction using a commercial RNA extraction kit (Vivantis, Malaysia), following the manufacturer's protocol. The tissue was homogenized on ice in a glass homogenizer. Approximately 300 µL of TRIzol-based lysis buffer was added to lyse the cells. The mixture was vortexed and centrifuged at 14,000×g for 3 minutes. The resulting lysate was transferred to a homogenization column within a collection tube and centrifuged at 14,000×g for 2 minutes. Subsequently, 650 µL of ethanol was mixed thoroughly using a pipette. For RNA isolation, the lysate was passed through an mRNA binding column fitted into a collection tube and centrifuged at 10,000×g for 1 minute. After column washing, DNase treatment was applied and incubated at room temperature for 15 minutes. Next, 500 µL of inhibitor removal solution was added and the column was washed twice. The RNA was finally eluted into a new tube using 50 µL of RNase-free water and centrifuged at 10,000×g for 1 minute. RNA concentration was determined using a Nanodrop spectrophotometer (ng/µL) and purity was assessed by measuring the A260/280 ratio. All RNA samples showed A260/280 values within the recommended range of 2.0 to 2.1, indicating high purity.

Complementary DNA (cDNA) synthesis was performed using the Viva cDNA synthesis kit (Vivantis, Malaysia), following the manufacturer's protocol. The components were mixed with nuclease-free water to a final volume of 10 µL. The mixture was incubated at 65°C for 5 minutes and then cooled on ice for 2 minutes. After a brief centrifugation, 10 µL of cDNA synthesis mix was added and the reaction was incubated at 42°C for 60 minutes. The reaction was terminated by heating at 85°C for 5 minutes, then by ice cooling. The resulting cDNA template was stored at −20°C until further analysis.

qPCR was performed using the ViPrimePLUS Taq qPCR Green Master Mix I (SYBR® Green Dye) kit (Vivantis, Malaysia), following the manufacturer's protocol. Each sample was run in triplicate. The target gene was LHCGR and the housekeeping genes used for normalisation were β-actin and Cyclin A2. The primer sequences and product sizes are listed in Table 1.

Primer efficiency was tested using fivefold serial dilutions to determine optimal sample dilution. Standard curves were generated and all primers' correlation coefficients (R^2) exceeded 0.98, with efficiencies ranging from 91 to 97%. qPCR was conducted for 45 cycles using the following thermal cycling conditions: initial enzyme activation at 95°C for 3 minutes, followed by denaturation at 95°C for 30 seconds and annealing at 53°C for 30 seconds. A melt curve analysis was performed from 53 to 95°C, increasing by 0.5°C every 5 seconds to verify product specificity. The final melt curve step was used to confirm the specificity of amplification by ensuring the presence of a single PCR product.

Threshold cycle (Ct) values were obtained at the end of each cycle based on fluorescence signals emitted by SYBR Green dye. The comparative Ct method (ΔΔCt) determined the relative gene expression of mRNA. First, the ΔCt for each sample was calculated by subtracting the average Ct of the housekeeping genes (β-actin and Cyclin A2) from the Ct of the target gene (LHCGR). Next, ΔΔCt was calculated by subtracting the Δcontrol group's Ct from the experimental sample's Ct. Fold change in gene expression for each sample was then calculated using the ΔΔCt method and converted to $\log_2$ fold change for standardization.

Measurement of LH Receptor Expression via Western Blot

Frozen testis tissue (0.3 g) was thawed and lysed using freshly prepared RIPA lysis buffer supplemented with protease inhibitors (PMSF and Na_3VO_4). All reagents were obtained from Elabscience, Wuhan, China.

Table 1: Primer Sequences and Product Sizes

Gene	Primer Sequence (5′–3′)	Product Size (bp)
LHCGR	Forward: TAT GCT CGG AGG ATG GCT CT Reverse: AGC ACA GAT GAC GAC GAA GG	175
β-actin	Forward: TAC AAC CTT CTT GCA GCT CCT Reverse: CCT TCT GAC CCA TAC CCA CC	205
Cyclin A2	Forward: TGG ATG GTA GTT TTG AAT CAC CC Reverse: TGG CCC GCA TAC TGT TAG TG	195

The tissue was homogenized on ice using a vortex mixer for 60 minutes to maintain protein integrity. The homogenate was then stored overnight at −80°C to improve protein yield. The following day, samples were thawed on ice and centrifuged at 12,000 rpm for 10 minutes at 4°C. The resulting supernatant, containing the extracted proteins, was collected for concentration measurement and further analysis.

Protein concentrations of the samples were determined using the Bradford assay, following the manufacturer's protocol. A two-fold serial dilution of the BSA standard stock solution (1mg/mL) was performed to generate eight concentration points. In a 96-well microplate, 250 µL of Bradford reagent was added to each well, followed by 5 µL of either standard or sample, in duplicate. After incubation at room temperature for 5 minutes, absorbance was measured at 595 nm using a microplate reader. A standard curve was generated by linear regression, producing a correlation coefficient (R^2) 0.973.

Protein separation was performed using SDS-PAGE. A 10% resolving gel and 5% stacking gel (Elabscience, Wuhan, China) were prepared following standard protocols. Cleaned glass plates with 1.00 mm spacers were assembled and mounted in a casting frame. After pouring the resolving gel, a thin layer of methanol (Merck, Germany) was applied to maintain a flat surface. Once polymerized, the methanol was removed and the stacking gel was added with a 1.00 mm comb inserted until solidification. Protein samples stored at −80°C were thawed and prepared by mixing 20 µg of protein with 5X SDS loading buffer (1:4 ratio), then denatured at 95°C for 10 minutes and centrifuged. Samples and a molecular weight ladder (MAestreogen, Taiwan) were loaded into the wells. Electrophoresis was performed at 120 V for 1 hour in 1X electrophoresis buffer (Elabscience, Wuhan, China), prepared by diluting a commercial 10X stock.

Following electrophoresis, proteins were transferred to a PVDF membrane using a wet transfer system. Membranes were activated in methanol, then equilibrated in transfer buffer containing methanol (Elabscience, Wuhan, China). The transfer sandwich was assembled in the order: fiber pad, filter paper, gel, membrane, filter paper, fiber pad. Air bubbles were removed before closing the cassette. Transfer was conducted at 40 V for 2 hours on ice.

After transfer, membranes were blocked with 5% skim milk in TBST (Elabscience, Wuhan, China) to reduce non-specific binding. Membranes were then incubated overnight at 4°C with primary antibody against LHCGR rabbit polyclonal, diluted 1:1000 in 5% skim milk (Signalway, Maryland, USA). After washing, membranes were incubated with HRP-conjugated goat anti-rabbit secondary antibody diluted 1:5000 in 2% skim milk (Elabscience, Wuhan, China). To normalize protein loading, a β-actin HRP-conjugated antibody (Elabscience, Wuhan, China) was used at 1:1000 dilution. Detection was performed using enhanced chemiluminescence and imaging was conducted with a gel documentation system. Band intensities of LHCGR were normalized against β-actin and densitometric analysis was performed using ImageJ software. Normalized values were then used for statistical comparisons between groups.

Statistical Analysis

The effects of different durations of RF-EMR Wi-Fi exposure (2.45 GHz) on serum reproductive hormones (LH and testosterone) and the expression of LH receptor in the testes were analyzed using one-way ANOVA followed by Tukey's post hoc test, provided the data showed a normal distribution (Kolmogorov-Smirnov test, $p>0.05$). Results were expressed as mean±SEM. For data that did not follow a normal distribution ($p<0.05$), the Kruskal-Wallis H test was used instead. A p-value of less than 0.05 was considered statistically significant for both ANOVA and Kruskal-Wallis tests.

RESULTS

Serum LH Level

The analysis showed no significant differences in LH serum level among the groups, with $F(3, 20) = 0.234$ and $p = 0.872$ (Figure 1).

Serum Testosterone Level

The analysis of serum testosterone level indicated no statistically significant differences among the groups, $H(3) = 5.460$, $p = 0.141$ (Figure 2). Mean rank values were 10.00 for the control group, 9.50 for Group 1, 18.00 for Group 2 and 12.50 for Group 3. While the differences were not statistically significant, there was a noticeable trend toward increased testosterone levels in the exposed groups compared to the control. The group with 8-hour exposure showed the highest mean rank, followed by those exposed for 24 and 4 hours.

LHCGR mRNA Expression

Figure 3 illustrates the relative expression of LHCGR mRNA across four experimental groups. The results revealed a significant difference in LHCGR mRNA expression among the groups following exposure to 2.45 GHz RF-EMR at different durations, $F(3, 18) = 52.617$, $p<0.001$. All exposure durations (4, 8 and 24 hours) increased LHCGR mRNA expression, with the highest expression observed in the 24-hour exposure group.

LHCGR Protein Expression

LHCGR protein expression (WB) was used to detect the initiation of the steroidogenesis process (Table 2).

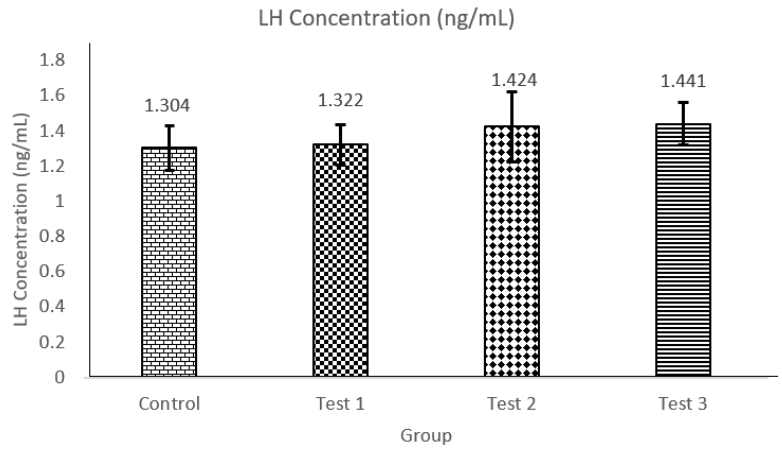

Figure 1: Serum LH Level for Each Experimental Group. Data are Presented as mean±SEM, with n = 6 per Group

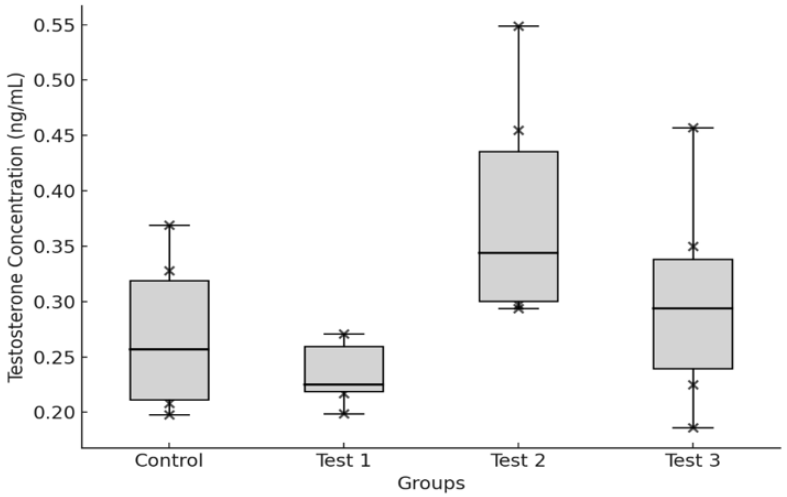

Figure 2: Serum Testosterone Concentration across Groups (n = 6 per Group). Data Shown as Box-and-Whisker Plots Indicating the Median, Interquartile Range (IQR), Whiskers and Individual Values. No Significant Differences were Observed between Groups (Kruskal-Wallis p = 0.141)

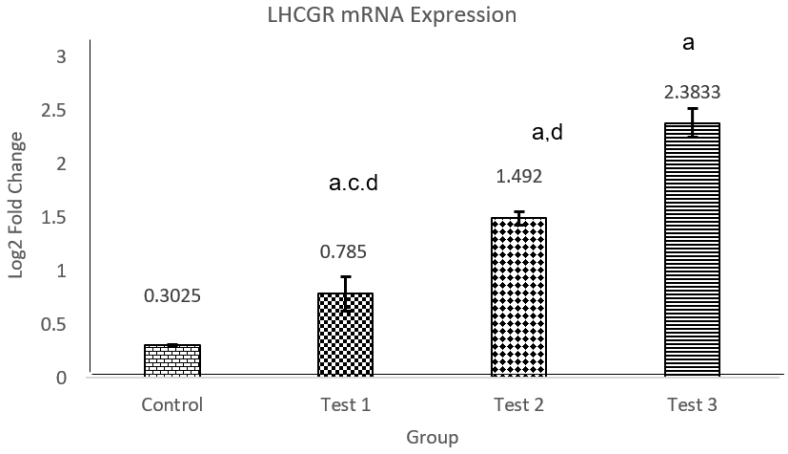

Figure 3: Log^2 fold Change of LHCGR mRNA Expression for each Experimental Group. Data are Presented as mean ± SEM, with n = 6 per Group. a Indicates a Significant Difference Compared to the Control group, b Suggests a Significant Difference Compared to Test 1, c Indicates a Significant Difference Compared to Test 2, and d Suggests a Significant Difference Compared to Test 3

Table 2: Effect of Different RF-EMR Wi-Fi (2.45 GHz) Exposure Durations on LHCGR Protein Expression (WB) in each Group

Group/ Protein	Control	Test 1	Test 2	Test 3
LHCGR (85kDa)				
β-aktin (43kDa)				

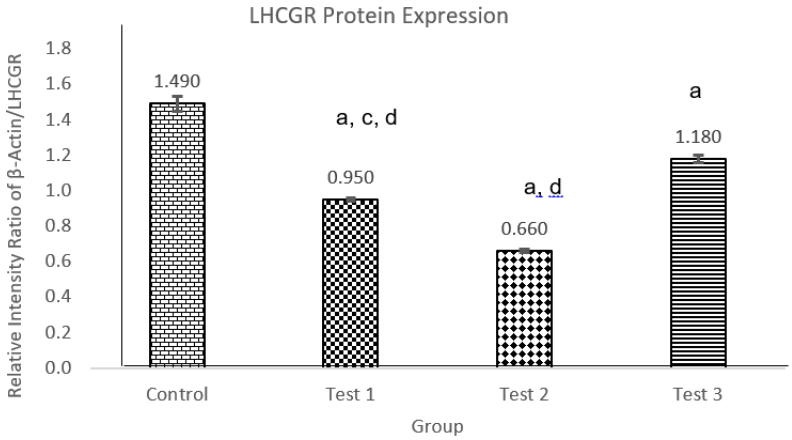

Figure 4: Relative Intensity Ratio of β-Actin/LHCGR Protein Expression for each Experimental Group. Data are Presented as mean±SEM, with n = 6 per Group. a Indicates a Significant difference Compared to the Control, b Suggests a Significant difference Compared to Test 1, c Suggests a Significant difference Compared to Test 2 and d Indicates a Significant difference Compared to Test 3

An analysis assessed the relative intensity ratio of β-Actin/LHCGR (Figure 4) across the four groups. A significant difference was observed among all groups, $F(3, 20) = 234.353$, $p<0.001$. Post hoc Tukey analysis revealed that Test 1, Test 2 and Test 3 were significantly different from the Control Group, $F(3, 20) = 234.353$, $p<0.001$. These findings suggest that 4-hour- and 8-hour exposures reduced the β-Actin/LHCGR intensity ratio, with the most significant decrease observed at 8 hours. In contrast, 24-hour exposure showed a recovery trend, with the ratio approaching near normal levels.

DISCUSSIONS

The results of this study demonstrated that serum concentrations of LH and testosterone (Figures 1 and 2) remained unchanged across all experimental groups. This out-come raises critical questions regarding the underlying mechanisms by which RF-EMR influences male reproductive health. One likely explanation is that 2.45 GHz RF-EMR exposure was not intense enough to disrupt the Hypothalamic-Pituitary-Gonadal (HPG) axis, which regulates the production of reproductive hormones. The hypothalamus releases GnRH, which stimulates the anterior pituitary to secrete LH. In turn, LH acts on Leydig cells in the testes to trigger testosterone production [11]. Previous reports showed that spermatogenic cells and seminiferous tubules can be vulnerable to RF-EMR exposure [26,27,28], whereas Leydig cells may exhibit greater resilience. This relative stability could account for the preserved systemic testosterone levels and the maintenance of LH feedback regulation observed here. Therefore, the consistency of hormone concentrations across exposure durations (4, 8 and 24 hours) suggests that the functional integrity of the hypothalamus-pituitary-testis axis was preserved [29].

Because the present study measured serum hormone levels rather than localized testicular conditions, the findings most likely reflect overall endocrine stability, even if localized testicular stress may occur. In the 4-hour exposure group, the short duration may have been insufficient to impair HPG axis function. At 8 and 24 hours, partial structural recovery in testicular tissue was evident, yet hormone synthesis remained unaffected [28]. This suggests that the RF-EMR-induced damage was not severe enough to reduce systemic testosterone concentrations. Consistent with this, [30] also reported no significant changes in testosterone levels following RF-EMR exposure, reinforcing the hypothesis that the biological effects of RF-EMR are more localized to the testes than systemic in nature.

These findings further challenge common concerns regarding RF-EMR exposure and its potential to lower testosterone levels, which could theoretically result in feminization or disrupt male development. On the contrary, testosterone levels remained sufficient to support normal reproductive and sexual function [12] and the hormonal regulatory axis appeared to be preserved. Interestingly, although not statistically significant, testosterone levels in exposed groups were higher than control group. This counterintuitive trend may indicate compensatory stimulation of steroidogenesis in response to cellular stress. One possible explanation is that RF-EMR may impair androgen-binding protein function [31], thereby prolonging the half-life of testosterone in the serum. However, this remains a postulation and warrants further investigation.

As LH and testosterone play central roles in steroidogenesis, we extended our analysis to examine how RF-EMR affects LHCGR expression, the receptor through which LH activates testosterone synthesis. LHCGR mRNA expression increased significantly with exposure time, with the highest levels observed at 24 hours (Figure 3). Meanwhile, LHCGR protein levels initially decreased at 4 and 8 hours before increasing again at 24 hours (Figure 4). This initial suppression of protein expression may have resulted from oxidative stress disrupting protein stability or synthesis, particularly in Leydig cells [28].

The early reduction in LHCGR protein suggests that RF-EMR-induced oxidative stress could impair protein synthesis efficiency within Leydig cells. This type of cellular stress has been previously shown to destabilize protein structure, interfere with post-translational modification and hinder receptor maturation [30]. The resulting changes in LHCGR expression may have temporarily reduced its ability to bind LH and transmit intracellular signals. However, the increase in LHCGR protein levels at 24 hours suggests that repair and recovery mechanisms had been activated. This late-stage rebound may represent a compensatory cellular strategy to restore LH sensitivity and maintain testosterone synthesis. This pattern suggests that RF-EMR initially disrupts Leydig cell function through oxidative pathways. Still, cellular repair systems may later compensate by upregulating receptor expression at the gene and eventually at the protein level.

Mechanistically, LHCGR expression is likely influenced by LH, testosterone and other regulatory factors. Once LH binds to LHCGR, it activates cAMP and protein kinase A (PKA), triggering steroidogenesis [32]. In addition, pathways such as Akt and ERK1/2, which support Leydig cell proliferation, survival and differentiation, may also contribute to the adaptive response observed in this study [26,29]**.**. The observed rebound of LHCGR protein at 24 hours may also involve MAPK activation, a pathway known to mediate adaptive responses to oxidative stress [33].

Moreover, growth factors such as Insulin-like Growth Factor (IGF) and epidermal growth factor (EGF) may also contribute to LHCGR regulation. IGF enhances LH receptor sensitivity and may help counteract the inhibitory effects of RF-EMR-induced oxidative damage [34]. EGF, which supports cell maintenance and growth, could also aid in Leydig cell adaptation during periods of stress [37]. These factors may promote Leydig cell recovery and sustained spermatogenesis following RF-EMR exposure. Additionally, prolactin, a hormone that influences testicular function, may regulate Leydig cell activity and interact with LHCGR expression during oxidative stress [35,33]. This suggests that RF-EMR may indirectly affect Leydig cell function through dynamic hormonal and growth factor changes within the testes.

Although both mRNA and protein expression of LHCGR were examined, we observed a notable mismatch. While mRNA levels increased with longer exposure durations, protein levels dropped before recovery. This discrepancy is not unexpected, as mRNA expression does not always directly correlate with protein output. Factors such as translation efficiency, protein degradation and molecule-specific half-lives contribute to temporal differences between transcript and protein levels [36-38]. Protein synthesis is also tightly regulated by complex post-transcriptional and translational mechanisms, including mRNA stability, translation initiation and protein turnover [28,30]. Differences in degradation and half-life further shape protein abundance [32]. These mechanisms could explain the observed increase in LHCGR mRNA alongside fluctuating protein expression. Thus, the temporal divergence between LHCGR mRNA and protein levels may reflect a typical cellular response to stress, where transcriptional upregulation is followed by delayed protein synthesis and accumulation.

CONCLUSION

In summary, our findings suggest that exposure to 2.45 GHz RF-EMR does not significantly alter systemic LH and testosterone levels, indicating that the overall HPG axis function was preserved under the conditions tested rather than being entirely unaffected. However, we observed early changes in LHCGR expression, particularly a drop in protein levels followed by partial recovery over time**,** while mRNA expression showed a progressive increase, highlighting a mismatch likely due to post-transcriptional regulation. This points to an adaptive response by Leydig cells to maintain hormone production under stress. Still, the function of exposed cells differed from controls in a measurable way, reflecting local gonadal effects. While RF-EMR appears to cause localized effects in testicular tissue, it does not seem to disrupt the hormonal balance necessary for normal male reproductive function. These results help ease concerns about the systemic effects of RF-EMR and highlight the body's ability to adapt at the cellular level. However, given the small sample size and reliance on serum rather than intratesticular hormone measurements, future studies should have a standardized dosimetry and direct assessment of testicular testosterone to strengthen mechanistic insight.

Acknowledgement

Financial support through Fundamental Research Grant Scheme (FRGS) (FRGS/1/2022/SKK06/UKM/03/2) is gratefully acknowledged. We extend our gratitude to the UKM University Library for providing access to the vital resources and databases utilized in this paper. Special thanks go to the staff and students of the Physiology Department and the Biochemistry Department (Noor Baitee A. Rahim), Faculty of Medicine, UKM, for their invaluable assistance. Lastly, we are profoundly grateful to our families and colleagues for their encouragement and understanding.

Funding

This research was funded by the Ministry of Higher Education (MOHE), Malaysia through Fundamental Research Grant Scheme (FRGS) under the grant number FRGS/1/2022/SKK06/UKM/03/2.

Author Contributions

Conceptualization, KO, SFI and FHFJ; Methodology, NJ; Software, KO and NJ; Validation, KO, SFI and FHFJ; Formal analysis, KO and NJ; Investigation, NJ, SMAST, AH, SV, AFZ. and MFMR; Resources, NJ; Data curation, KO and NJ; Writing-original draft preparation, NJ; Writing-review and editing, KO, FHFJ and SFI.; Supervision, KO, SFI and FHFJ; Project administration, AFZ; Funding acquisition, KO. All authors have read and agreed to the published version of the manuscript.

Ethical Statement

The animal study protocol was approved by the UKM Animal Ethics Committee, with the approval number FSK/2022/KHAIRULOSMAN/20-JULY/1262-AUG.-2022-AUG.-2024.

REFERENCES

[1] Agarwal, A. *et al.* "Effect of cell phone usage on semen analysis in men attending infertility clinic: An observational study." *Fertility and Sterility*, vol. 89, no. 1, 2008, pp. 124–128. https://doi.org/10.1016/j.fertnstert.2007.01.166

[2] SKKM. *Internet User Survey 2020 – Infographic.* 2020. https://mcmc.gov.my/en/resources/statistics/internet-users-survey/internet-users-survey-2020

[3] Andric, S.A. and T.S. Kostic. "Regulation of Leydig cell steroidogenesis: An intriguing network of signalling pathways and mitochondrial signalosome." *Current Opinion in Endocrine and Metabolic Research*, vol. 6, 2019, pp. 7–20. https://doi.org/10.3390/endocrines4030041

[4] Aneja, A. and G. Sodhi. "A study of security issues related to wireless fidelity (Wi-Fi)." *International Journal of Computer Science Trends and Technology*, vol. 4, no. 2, 2016.

[5] Bakhtyukov, A. *et al.* "Thienopyrimidine derivatives specifically activate testicular steroidogenesis but do not affect thyroid functions." *Journal of Evolutionary Biochemistry and Physiology*, vol. 55, 2019, pp. 30–39. https://doi.org/10.1134/S0022093019010046

[6] Creasy, D.M. and R.E. Chapin. "Male reproductive system." *Haschek and Rousseaux's Handbook of Toxicologic Pathology*, 2013, pp. 2493–2598.

[7] Darbandi, M. *et al.* "Reactive oxygen species and male reproductive hormones." *Reproductive Biology and Endocrinology*, vol. 16, no. 1, 2018, p. 87. https://doi.org/10.1186/s12958-018-0406-2

[8] De Mattos, K. *et al.* "Hormones and signaling pathways involved in the stimulation of Leydig cell steroidogenesis." *Endocrines*, vol. 4, no. 3, 2023, pp. 573–594. https://doi.org/10.3390/endocrines4030041

[9] Djeridane, Y. *et al.* "Influence of electromagnetic fields emitted by GSM-900 cellular telephones on the circadian patterns of gonadal, adrenal and pituitary hormones in men." *Radiation Research*, vol. 169, no. 3, 2008, pp. 337–343. https://doi.org/10.1667/RR0922.1

[10] Zhou, H.D. *et al.* "Effects of 1.5-GHz high-power microwave exposure on the reproductive systems of male mice." *Electromagnetic Biology and Medicine*, vol. 40, no. 2, 2021, pp. 311–320. https://doi.org/10.1080/15368378.2021.1891091

[11] Dongus, S. *et al.* "Health effects of WiFi radiation: A review based on systematic quality evaluation." *Critical Reviews in Environmental Science and Technology*, vol. 52, no. 19, 2021, pp. 3547–3566. https://doi.org/10.1080/10643389.2021.1951549

[12] Grande, G. *et al.* "The role of testosterone in spermatogenesis: Lessons from proteome profiling of human spermatozoa in testosterone deficiency." *Frontiers in Endocrinology*, vol. 13, 2022, p. 852661. https://doi.org/10.3389/fendo.2022.852661

[13] Oyedokun, P. *et al.* "Impact of hypoxia on male reproductive functions." *Molecular and Cellular Biochemistry*, vol. 478, no. 4, 2023, pp. 875–885. https://doi.org/10.1007/s11010-022-04559-1

[14] Ozguner, M. *et al.* "Biological and morphological effects on the reproductive organ of rats after exposure to electromagnetic field." *Saudi Medical Journal*, vol. 26, no. 3, 2025, pp. 405–410.

[15] Yahyazadeh, A. *et al.* "Biochemical, immunohistochemical and morphometrical investigation of the effect of thymoquinone on the rat testis following exposure to a 900-MHz electromagnetic field." *Acta Histochemica*, vol. 122, no. 1, 2020, p. 151467. https://doi.org/10.1016/j.acthis.2019.151467

[16] Farag, E. *et al.* "Histological study on the detrimental influences of white LED light on the retina of adult albino rat and the potential effect of simultaneous nicotine administration with highlighting their possible mechanisms." *Egyptian Journal of Histology*, vol. 40, no. 3, 2017, pp. 328–344. https://doi.org/10.21608/EJH.2017.4659

[17] El-Bediwi, A. *et al.* "Effects of electromagnetic radiation produced by mobile phone on some visceral organs of rat." *Journal of Medical Sciences*, vol. 11, no. 6, 2011, pp. 256–260. https://doi.org/10.3923/jms.2011

[18] Djeridane, Y. *et al.* "Influence of electromagnetic fields emitted by GSM-900 cellular telephones on the circadian patterns of gonadal, adrenal and pituitary hormones in men." *Radiation Research*, vol. 169, no. 3, 2018, pp. 337–343. https://doi.org/10.1667/RR1053.1

[19] Shahin, N.N. *et al.* "The protective role of spermine against male reproductive aberrations induced by exposure to electromagnetic field – An experimental investigation in the rat." *Toxicology and Applied Pharmacology*, vol. 370, 2019, pp. 117–130. https://doi.org/10.1016/j.taap.2019.04.008

[20] Meo, S.A. *et al.* "Effects of mobile phone radiation on serum testosterone in Wistar albino rats." *Saudi Medical Journal*, vol. 31, no. 8, 2010, pp. 869–873.

[21] Qin, F. *et al.* "1800 MHz radiofrequency fields inhibits testosterone production via CaMKI/RORα pathway." *Reproductive Toxicology*, vol. 81, 2018, pp. 229–236. https://doi.org/10.1016/j.reprotox.2018.08.007

[22] Saygin, M. *et al.* "Impact of 2.45 GHz microwave radiation on the testicular inflammatory pathway biomarkers in young rats: The role of gallic acid." *Environmental Toxicology*, vol. 31, no. 12, 2016, pp. 1771–1784. https://doi.org/10.1002/tox.22179

[23] Ibrahim, R.A. *et al.* "Effect of exposure to Wi-Fi router radiation on the lung of adult male albino rats: Histological and immunohistochemical study." *Egyptian Journal of Histology*, vol. 42, no. 4, 2019, pp. 1059–1069. https://doi.org/10.21608/ejh.2019.7317.1070

[24] Jamaludin, N. *et al.* "The influence of 2.45 GHz Wi-Fi exposure duration on sperm quality and testicular histopathology: An exploration of peroxidative injury." *Antioxidants*, vol. 14, no. 2, 2025, p. 179. https://doi.org/10.3390/antiox14020179

[25] Jaffar, F.H.F. *et al.* "Long-term Wi-Fi exposure from pre-pubertal to adult age on the spermatogonia proliferation and protective effects of edible bird's nest supplementation." *Frontiers in Physiology*, vol. 13, 2022, p. 828578. https://doi.org/10.3389/fphys.2022.828578

[26] Oyewopo, A.O. *et al.* "Radiofrequency electromagnetic radiation from cell phones causes defective testicular function in male Wistar rats." *Andrologia*, vol. 49, no. 10, 2017, p. e12772. https://doi.org/10.1111/and.12772

[27] Raut, S. *et al.* "Unveiling the role of prolactin and its receptor in male reproduction." *Hormone and Metabolic Research*, vol. 51, no. 4, 2019, pp. 215–219. https://doi.org/10.1055/a-0859-1144

[28] Richer, G. *et al.* "*In-vitro* spermatogenesis through testis modelling: Toward the generation of testicular organoids." *Andrology*, vol. 8, no. 4, 2020, pp. 879–891. https://doi.org/10.1111/andr.12741

[29] Sepehrimanesh, M. *et al.* "The impact of 900 MHz electromagnetic field exposure on primary male reproductive hormone levels in a Rattus norvegicus model." *International Journal of Biometeorology*, vol. 58, no. 7, 2014, pp. 1657–1663. https://doi.org/10.1007/s00484-013-0771-7

[30] Hoernes, T.P. *et al.* "mRNA modifications: Dynamic regulators of gene expression?" *RNA Biology*, vol. 13, no. 9, 2016, pp. 760–765. https://doi.org/10.1080/15476286.2016.1203504

[31] Vogel, C. and E.M. Marcotte. "Insights into the regulation of protein abundance from proteomic and transcriptomic analyses." *Nature Reviews Genetics*, vol. 13, no. 4, 2012, pp. 227–232.

[32] Shokri, M. *et al.* "The protective effect of melatonin on radiofrequency electromagnetic fields of mobile phone-induced testicular damage in an experimental mouse model." *Andrologia*, vol. 52, no. 11, 2020, p. e13834. https://doi.org/10.1111/and.13834

[33] Shpakov, A.O. "Hormonal and allosteric regulation of the luteinizing hormone/chorionic gonadotropin receptor." *Frontiers in Bioscience – Landmark*, vol. 29, no. 9, 2024, p. 313. https://doi.org/10.31083/j.fbl2909313

[34] Sundaram, V. *et al.* "Effects of 150 kHz intermediate frequency electromagnetic radiation on fertility indicators in male rats." *Heliyon*, vol. 8, no. 12, 2022, p. e12228. https://doi.org/10.1016/j.heliyon.2022.e12228

[35] Li, Y. *et al.* "1,800 MHz radiofrequency electromagnetic irradiation impairs neurite outgrowth with a decrease in Rap1-GTP in primary mouse hippocampal neurons and neuro2a cells." *Frontiers in Public Health*, vol. 9, 2021, p. 771508. https://doi.org/10.3389/fpubh.2021.771508

[36] Xue, S. *et al.* "Effects and mechanisms of endocrine disruptor bisphenol AF on male reproductive health: A mini-review." *Ecotoxicology and Environmental Safety*, vol. 276, 2024, p. 116300. https://doi.org/10.1016/j.ecoenv.2024.116300

[37] Yahyazadeh, A. and B.Z. Altunkaynak. "Protective effects of luteolin on rat testis following exposure to 900 MHz electromagnetic field." *Biotechnic & Histochemistry*, vol. 94, no. 4, 2019, pp. 298–307. https://doi.org/10.1080/10520295.2019.1566568

[38] Zirkin, B.R. and V. Papadopoulos. "Leydig cells: Formation, function and regulation." *Biology of Reproduction*, vol. 99, no. 1, 2018, pp. 101–111. https://doi.org/10.1093/biolre/ioy059